User manual

Check-Direct CPE for SmartCycler

Real-time PCR kit for the detection of carbapenemase-producing *Enterobacteriaceae*

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Not for use in diagnostic procedures

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Intended use

Check-Direct CPE is a qualitative *in vitro* diagnostic test for the rapid detection of carbapenemase genes in *Enterobacteriaceae*. At present the test is validated for bacteria cultured from various clinical specimens. Check-Direct CPE detects the presence of the carbapenemase genes KPC, NDM, VIM, and OXA-48, presently the primary cause of carbapenemase production in *Enterobacteriaceae*. The assay involves the extraction of DNA from bacterial cells followed by real-time PCR using the reagents provided with the kit. Check-Direct CPE can be used as an aid to identify, prevent and control carbapenemase-producing *Enterobacteriaceae* that colonize patients in healthcare settings. Check-Direct CPE is not intended to diagnose infections with carbapenemase-producing *Enterobacteriaceae* nor to guide or monitor treatment for these infections. Parallel cultures are necessary to recover organisms for epidemiological typing, susceptibility testing and for further confirmatory identification.

Introduction

The worldwide emergence and dissemination of carbapenem resistance among *Enterobacteriaceae* is a serious threat to public health. These organisms are associated with high mortality rates and have the potential to spread widely. The most common cause of carbapenem resistance in *Enterobacteriaceae* is the expression of carbapenemases, *i.e.* Carbapenemase-Producing *Enterobacteriaceae* or CPE. CPE have elevated or complete resistance to carbapenems and most other β-lactam antibiotics. Presently, the vast majority of CPE are associated with the presence of one of the following plasmid-encoded carbapenemases: KPC (*Klebsiella pneumoniae* carbapenemase), VIM (Verona integron–encoded metallo-β-lactamase), NDM (New Delhi metallo-β-lactamase) or OXA-48 (Oxacillinase-48). Moreover, CPE often have other non-β-lactam resistance determinants resulting in multidrug- and pandrug-resistant isolates.

Check-Direct CPE is a rapid real-time PCR test for the detection and discrimination of KPC, NDM/VIM and OXA-48.

Principle of the method

Check-Direct CPE assay is based on specific recognition and amplification of target sequences by PCR, and the simultaneous detection of the accumulation of PCR amplification products by fluorescent DNA probes. A control DNA molecule, the internal control, is added to the clinical specimen prior to DNA extraction to monitor that DNA extraction and PCR amplification were successful. Five molecular beacon probes, labeled with 4 different dyes are used to detect the various carbapenemases and the control DNA. Check-Direct CPE discriminates between KPC, NDM/VIM, and OXA-48. For each of the 4 carbapenemase genes, KPC, OXA-48, NDM and VIM, many gene variants exist. PCR primers and fluorescent probes of Check-Direct CPE are selected to target homologous gene segments of these carbapenemase genes, and in this way gene variants are reliably detected.

Kit contents (for 48 reactions)

Components (Mat. No.)	Description	Storage conditions
CPE PCR Mastermix (9-0080)	1 transparent tube and cap 660µl	+ 4°C
CPE solution (9-0081)	1 brown tube (blue inlay 🔵) 140 µl	- 20°C, **store in the dark**
Internal Control (IC) (9-0107)	1 tube (red inlay 🔴) 600 µl	
Negative control (9-0100)	1 tube (white inlay ○) 100 µl	
KPC positive control (9-0083)	1 tube (green inlay 🟢) 100 µl	
NDM positive control (9-0085)	1 tube (gold inlay 🟡) 100 µl	
VIM positive control (9-0084)	1 tube (yellow inlay 🟡) 100 µl	
OXA-48 positive control (9-0086)	1 tube (orange inlay 🟠) 100 µl	
User manual (9-0088)	Leaflet – download from website	-

Materials required but not supplied with the kit

Supplies	Equipment
• SmartCycler 25 µl Reaction Tubes, cat no 900-0003 or 900-0022 • Disposable laboratory (powder-free) gloves/Lab coat • Pipettes & disposable (filter-) tips for volumes of 10 to 1000 µl • PCR-grade water (e.g. Milli-Q or aqua bidest) • 1.5 ml tubes (“Eppendorf tubes”)	• Real-time PCR instrument: Cepheid SmartCycler 2.0, software version 2.0d • Densitometer suitable for bacterial suspensions • Mini-centrifuge with rotor for SmartCycler 25 µl Reaction Tubes cat no 900-0021 • SmartCycler Tube Rack, cat no 300-0276 • Vortex mixer

Storage, handling, and stability

Check-Direct CPE reagents are shipped cooled. The CPE PCR Mastermix should be stored at +4°C upon receipt. All other reagents should be stored at -20°C upon receipt. Please visually inspect the box upon initial opening to ensure that its contents are intact. The CPE solution should not be exposed to more than 12 freeze-thaw cycles. Please contact the Check-Points office at *support@check-points.com* if you have any further questions. Store kit reagents at indicated temperature until expiration date.

Good laboratory practices

Recommendations for best results

- The test must be performed by adequately trained personnel.
- Do not use reagents after their expiration date.
- Before use, thaw frozen reagents completely at room temperature and vortex briefly to obtain a homogeneous solution. After vortexing briefly, spin down the solution to avoid contamination when opening the cap.
- Follow recommendations for storage, handling and freeze-thaw cycles to preserve the quality of the kit's reagents.
- Protect reagents from light to avoid photo-bleaching of the dyes.
- Periodically, verify the accuracy and precision of pipettes, as well as correct functioning and calibration of the instruments.

Prevention of contaminations

Use separate rooms: a pre-PCR room and a PCR room.

- DNA extraction and preparation of the amplification reactions are carried out in the pre-PCR room.
- Incubation in the real-time PCR thermocycler is carried out in the PCR room.
- Never transfer items from the PCR room to the pre-PCR room.

To keep laboratory free of PCR product contamination:

- Use pipettes with hydrophobic filter tips.
- Make sure to always use a new pipette tip when adding solutions, test samples, and controls to wells of a 96-well plate.
- Follow proper pipette-dispensing techniques to prevent aerosols.
- Wear clean disposable gloves and clean lab coats for the different steps of the test.
- Change gloves whenever you suspect that they are contaminated.
- Keep the tubes of all kit components and samples closed as much as possible.
- Clean the lab benches and all equipment regularly with a 0.5% sodium hypochlorite solution.

Users are strongly advised to read the full protocol before starting the test

Specimen collection and DNA extraction

Crude DNA extraction from bacterial cells

Important points before starting: DNA extraction is carried out in the pre-PCR room.

Procedure:

1. Inoculate nutrient agar plates with the clinical samples or the bacterial strains to be tested and incubate overnight at 37°C. Typical growth media include blood agar, MacConkey agar and Tryptic Soy agar. Bacteria from selective chromogenic plates may also be used.
2. Prepare a bacterial cell suspension of McFarland 0,5 – 1,0 or OD_{600} 0,08 – 0,15 using PCR-grade water (e.g. Milli-Q or bidest water).
3. For each cell suspension, transfer 200 µl to a 1.5 ml Eppendorf tube (preferably safe lock) and add 10 µl of the internal control solution (IC solution, ●). Mix briefly.
4. Heat the tubes at 98°C for 10 minutes. After incubation vortex the tubes vigorously for 30 seconds.
5. Centrifuge the tubes in an Eppendorf centrifuge for 2 minutes at maximum speed.
6. Use the supernatant immediately or store at +4°C and use within 24 hours. Alternatively, stored at -20°C for longer periods.

Positive and negative control preparation

To validate the run, perform positive and negative control reactions for each Check-Direct CPE PCR run. The positive and negative controls are supplied with the kit.

- **Positive control(s)**
  One positive control per target is provided with the kit. Each positive control contains the internal control. These controls may be used individually or combined. Refer to step 2.4 of the *Real-Time PCR preparation* for further details.

- **Negative control(s)**
  Use the negative control (○) provided in the kit as a sample to validate the run. The negative control contains the internal control. We also recommend performing a DNA extraction as specified earlier, with the internal control solution using a sample known to be negative for the test in use (*i.e.*, carbapenemase negative sample, or elution buffer).

Real-time PCR assay and SmartCycler Operation

1. Multiplex real-time PCR setup

Table 1 presents the multiplex real-time PCR setup with the targets detected in each detector channel of the SmartCycler System.

Table 1: *SmartCycler multiplex real-time PCR setup*

Detector	FAM	Cy3	Texas Red	Cy5
Channel	1	2	3	4
Target	KPC	VIM/NDM	OXA-48-like	I.C.*

*I.C: Internal Control

When the test is performed for the first time create the PCR test program “Check-Direct CPE” as described in Appendix 1.

2. Real-time PCR preparations

2.1 Calculate the number of reactions. Thaw reagents, mix well, spin down and keep on ice.

2.2 Prepare the real-time PCR mix as described in Table 2. Multiply the CPE solution and the CPE PCR Mastermix by the right number of samples and include 10% surplus to ensure that you have enough reaction mix for all the calculated reactions.

2.3 Pipette 15 µl of qPCR reaction mix to each of the SmartCycler Reaction Tubes, i.e. Smart-Tubes.

2.4 Pipette 10 µl of test sample or control sample to each pre-filled Smart-Tube. (The 4 positive controls may also be combined into a single "mixed positive control" by adding equal volumes of each control up to a volume of 10 µl per reaction, i.e. 4 x 2.5 µl of each control).

2.5 Close the Smart-Tubes and centrifuge for minimum 7 seconds. Check that no air bubbles have accumulated in the optical part of the tubes (see SmartCycler®II Operator Manual). Place the tubes in the SmartCycler Tube Rack and transfer the tubes to the PCR room.

Table 2: *real-time PCR reaction mix setup.*

Component	Volume per reaction
CPE PCR Mastermix	12,5 µl
CPE Solution (●)	2,5 µl
Total volume	15 µl

3. SmartCycler Operation

3.1 Insert the Smart-Tubes into the required PCR "Sites" (reaction chambers) of the SmartCycler. Press the tubes firmly and close each PCR Site.

3.2 Click "Create Run" and specify "Run Name". If needed add remarks in "Notes".

3.3 Select the Dye Set: "FCTC25".

3.4 Click the "Add/Remove Sites" button. A new window "Select Protocols and Sites" will be displayed in which you need to specify the "Protocol" and the number of PCR "Sites" for the test procedure. Select "Check-Direct CPE" as the "Protocol" (See Appendix 1 if not specified). Highlight the "Sites" in use for the test procedure and press the right arrow. When the Protocol and Sites have been selected click "OK".

3.5 Enter the sample I.D.'s for every site in the Sample ID column.

3.6 Click the "Start Run" button.

3.7 When the run is completed, discard the Smart-Tubes according to local regulations.

Results interpretation

Important points before starting: For a detailed description on how to analyze data, refer to *SmartCycler System User's manual*. Default settings should be used for analysis of Check-Direct CPE results. Refer to Appendix 2.
Always visually inspect the amplification plot for each sample tested versus C_T values obtained with the software.

1. Reported results

The SmartCycler software reports Result, C_T value and amplification curves for each detector channel of each specimen tested. Results are reported in the "Result table" of the "Views" window in the following way:

- NEG indicates no amplification was detected for that detector channel, i.e. FAM, Cy3, TxR and Cy5. C_T value of NEG result = 0.00. Amplification curves of samples showing a NEG result and 0.00 C_T value must be checked visually. For this purpose click on the "FAM", "Cy3", "TxR" or "Cy5" Tab in de "Views" window.
- POS indicates that amplification was detected for that detector channel, i.e. FAM, Cy3, TxR and Cy5. POS result will indicate a certain C_T value. Amplification curves of samples showing a POS result should also be confirmed visually and checked for abnormalities. For example see Figure 1.
- C_T values should be interpreted in correlation with the amplification curves and according to the interpretation method outlined in Tables 3 and 4.

2. Interpretation

2.1 Run validation

Check the positive and negative control amplification curves. Valid runs:

- No instruments system failures during the run.
- Positive and Negative Controls are within the C_T values specified in **Table 3**.

If the C_T values of the controls are not as expected refer to FAQ and Troubleshooting **"3"**.

Table 3: *Criteria for a valid run with Check-Direct CPE test.*

Controls / Expected C_T values	KPC FAM	NDM Cy3	VIM Cy3	OXA-48 TxR	IC Cy5
Positive controls Separate	30 ±3	31 ±3	29 ±3	27 ±3	35 ±3
Positive controls Mixed	33 ±3	30 ±3	30 ±3	30 ±3	34 ±3
Negative control	0.00	0.00	0.00	0.00	35 ±3

2.2 Results interpretation

If the run is valid, interpret results as positive, negative or invalid with the C_T values obtained for the samples with the guidelines summarized in **Table 4**.

- Positive carbapenemase samples will show a C_T value in the FAM, Cy3 and/or TxR channel. Positive carbapenemase samples will also show a C_T value in the Cy5 channel if the target has not out competed the internal control (IC) during the reaction. Always visually inspect the amplification plot to verify the result. See Figure 1 as an example.
- Negative carbapenemase samples will show no C_T value in the FAM, Cy3, and TxR Channel. In the Cy5 Channel, a C_T value is expected at 35 ±3.
- Samples with a FAM, Cy3 and TxR C_T-value of 0.00 (NEG) and with a Cy5 C_T value of 0.00 or > 38 indicate that the sample run is invalid, see FAQ and Troubleshooting 2 to 6.

Table 4: *Data interpretation guidelines*.*

KPC, NDM/VIM, OXA-48 C_T values	IC C_T values	Interpretation
YES	YES or 0.00	Positive sample
0.00	35 ±3	Negative sample
0.00	0.00 or >38	Invalid

* If observed C_T values vary significantly from expected C_T values , see FAQ and Troubleshooting section.

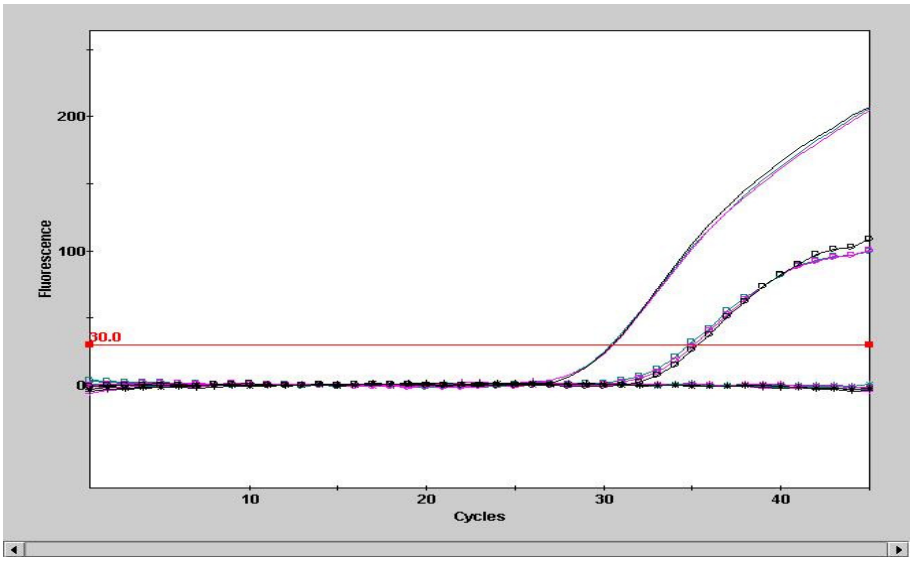

Figure 1: *Screenshot of KPC positive sample run in triplicate on the SmartCycler system.*

Frequently asked questions (FAQ) & Troubleshooting

1. **May other specimen preparation and DNA extraction methods be used with Check-Direct CPE?**
   Check-Direct CPE test has been optimized using specific swabs and transport medium in combination with the NucliSENS® easyMAG® extraction methods. The crude DNA extraction method from bacterial cells specified in this User Manual may also be used. Check-Points does not guarantee the performance of the test with methods other than those recommended in this manual.

2. **The real-time results show no C_T values or interpretation indicating that the sample is invalid.**
   Possible causes and troubleshooting:
   - The sample DNA was not added to the assay.
   - The sample DNA tested with Check-Direct CPE is negative and the internal control was not added prior to DNA extraction. Please repeat the DNA extraction.
   - The DNA extraction failed since the internal control was not detected. Please repeat the DNA extraction.
   - The sample DNA contains contaminants inhibiting the reactions. Please repeat the DNA extraction.
   - CPE Solution or CPE PCR Mastermix was not added to the assay. Please repeat the test.
   - Reagent solutions are degraded or may have expired.

3. **The real-time results show no C_T-values for the positive control or interpretation indicating that sample is invalid.**
   Possible causes and troubleshooting:
   - The positive control solution was not added. Repeat the test.
   - CPE Solution or CPE PCR Mastermix was not added to the assay. Please repeat the test.
   - Reagent solutions are degraded or may have expired.

4. **Troubleshooting for invalid result.**
   Repeat the test using the same DNA extract. If invalid results persist, repeat the test by preparing a new DNA extract from the original specimen. Alternatively, repeat the test with a new DNA extraction from a newly collected specimen.

5. **Real-time results show very low fluorescent signals in all samples and detector channels, including the internal control signal.** Possible causes and troubleshooting:
   - The CPE solution containing the fluorescent probes and primers is degraded. Please check expiration date, the number of thaw/freezing cycles that the CPE solution tube has undergone, and if the kit was stored correctly.
   - The real-time PCR system may be responsible for these results. Please refer to instrument User's manual or contact your real-time PCR instrument local representative.

6. **C_T values troubleshooting.**
   - ***Samples with very low C_T values and no amplification curves.*** The manual threshold is too low. Samples showing a C_T value <15 and no amplification curve are considered negative. The observed C_T value is an artifact of the software analysis: the threshold crosses the background noise of the curve.
   - ***Invalid Run: C_T values expected for the controls in Table 6 do not match the C_T values observed*** **In the experiment.** Check that these differences are not due to the threshold being too high or too low. If changing the threshold does not improve the results, go to FAQ **3. to 6.**

7. **The real-time PCR instrument gives an error message.**
   Refer to the real-time PCR instrument user manual or contact the local technical support of the real-time PCR instrument company.

8. **I left Solutions (CPE, Internal control, negative, or positive control) out of the -20°C (-4˚F) storage.**
   These reagents must be stored at -20°C (-4°F) for proper performance of the test. The performance of the product cannot be fully guaranteed if these solutions were left out of -20°C (-4˚F) for more than 24 hours.

9. **Duplicate samples tested with Check-Direct CPE test did not yield identical results.**
   C_T values of identical samples may vary between individual reactions. Large variations, > 2 C_T values, suggest pipetting errors or other differences between the duplicate samples.

10. **Data Analysis and Interpretation.**
    If you encounter difficulties with the data analysis and interpretation please contact Check-Points Technical Support at *support@check-points.com*.

Limitations

Check-Direct CPE is a DNA-based real-time PCR assay to detect the presence of the carbapenemase genes KPC, NDM, VIM, and OXA-48 in *Enterobacteriaceae*. The test detects the following carbapenemase gene variants: VIM1-6 & 8-38; OXA-48-162-163-181-232-244-245-247-370; NDM1-10 and KPC1-17. KPC, NDM, VIM, and OXA-48 represent the clinically most prevalent carbapenemases in *Enterobacteriaceae* in most parts of the world. However, other rare carbapenemases may also be responsible for carbapenemase production in *Enterobacteriaceae* and these are not detected by Check-Direct CPE. Carbapenem resistance is caused by carbapenemase production, but also by various other mechanisms. A negative result with Check-Direct CPE does not imply that the bacterium is not carbapenem resistant; it implies that the bacterium is not likely to carry any of the carbapenemase gene variants of KPC, NDM, VIM and OXA-48 detected by Check-Direct CPE. Therefore, the test result of Check-Direct CPE should never be used as guidance for therapy.

The quality of the input DNA is an important factor for obtaining reliable results with Check-Direct CPE. This User Manual describes the use of crude DNA extracts from bacterial cells. This will only work properly using pure water, i.e. MilliQ or aqua bidest, and the specified amount of cells. Other buffers or saline solutions will yield poor results. Other DNA extraction methods have not been tested with Check-Direct CPE for SmartCycler and may yield poor results.

The assay has been tested extensively with samples containing various gram-negative bacteria, such as *Escherichia*, *Salmonella*, *Klebsiella*, *Enterobacter*, *Citrobacter* and *Pseudomonas*, with excellent results. However, it may never be excluded that other Gram-negative bacteria or certain strains of the above species will yield poor results. Check-Direct CPE cannot and does not make any representation or warranty that it is capable of correctly detecting KPC, NDM, VIM, and OXA-48 in all gram-negative species, subspecies or types or in all clinical sample sources. Results may need to be confirmed by additional methodologies in specific cases (*e.g.* for regulatory samples). Due to the high variability of bacterial genomes it is possible that certain subtypes might not be detected. The test reflects the state of knowledge of Check-Points Health B.V.

As with other diagnostic assays, the results of this test may only be interpreted in combination with additional laboratory and clinical data available to the responsible person. Use of this assay is limited to appropriately qualified personnel, well-trained in performing DNA-based molecular detection methods.

Key to symbols used

Symbol	Definition	Symbol	Definition
IVD	For *In Vitro* Diagnostic Use	CONTROL –	Negative control
REF	Catalog number	CONTROL KPC	KPC positive control
LOT	Batch code	CONTROL VIM	VIM positive control
IFU	IFU number	CONTROL NDM	NDM positive control
	Use before YYYY-MM	CONTROL OXA-48	OXA-48 positive control
	Consult instructions for use	INTERNAL CONTROL	Internal control
	Manufacturer	SOLUTION CPE	CPE solution
	Temperature limitation	PCR Mastermix CPE	CPE PCR Mastermix
	Contains sufficient for < n > tests		

Technical assistance

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Despite the utmost care in the development and preparation of the protocol Check-Points cannot take any responsibility for errors, omissions and/or future changes herein.
Literature Citation: When describing a procedure for publication using this product, please refer to it as the *Check-Direct CPE.*
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Check-Points

Appendix 1: Create the Check-Direct CPE protocol

Important points before starting: Refer to SmartCycler System User's Manual for detailed instructions on how to operate the SmartCycler System and software version 2.0d.

1. Open the SmartCycler software and click "Define Protocols".
2. Click "New Protocol" (bottom left).
3. Enter Name "Check-Direct CPE".
4. Enter the parameters for the Check-Direct CPE protocol as outlined in Figure A below.
5. When ready click "Save Protocol".

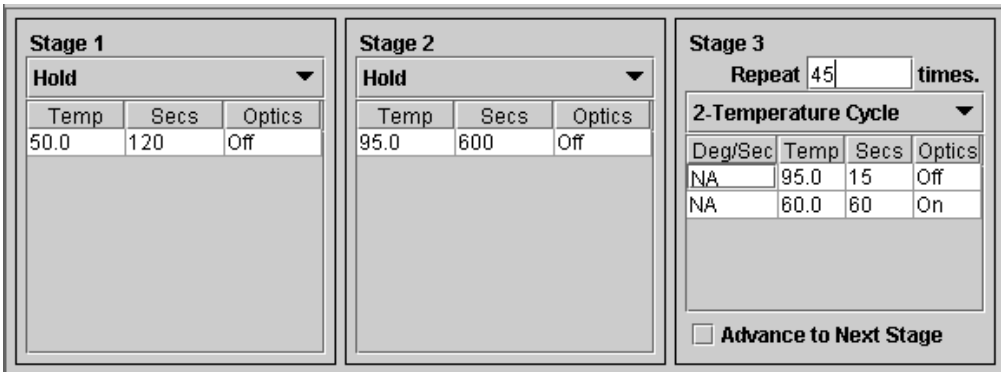

Figure A : *Real-time protocol parameters.*

Appendix 2: Default settings for analysis of Check-Direct CPE results

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle	Curve Analysis	Thresh Setting	Manual Thresh Fluor Units	Auto Thresh #SD's	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Boxca Cyc
1	FAM	Assay	ON	5	40	Primary Curve	Manual	30.0	NA	5	10	3	60	0
2	Cy3	Assay	ON	5	40	Primary Curve	Manual	30.0	NA	5	10	3	60	0
3	TxR	Assay	ON	5	40	Primary Curve	Manual	30.0	NA	5	10	3	60	0
4	Cy5	Assay	ON	5	40	Primary Curve	Manual	30.0	NA	5	10	3	60	0

Appendix 3: Performance Characteristics

1. Limit of Detection

The analytical limit of detection (LoD) of Check-Direct CPE was determined using the individual positive controls supplied with the test. These positive controls contain the target DNA at 10^3 copies per µl. Serial dilutions were made of each of the positive controls and 10^4, 10^3, 10^2 and 10 copies were added in quadruplicate: a total of 64 reactions following the protocol as described on pages 4 and 5 of this User Manual.

The 10^4, 10^3 and 10^2 DNA copies were always detected regardless of the target DNA used. Only at 10 copies added as input DNA for the test a difference in LoD was visible, which is depicted in the table A.

Table A

Target	Input DNA	Success
KPC	10	4 out of 4
VIM	10	4 out of 4
OXA-48	10	4 out of 4
NDM	10	2 out of 4

2. Specificity

2.1 *In silico* Specificity

The specificity of the Check-Direct CPE real-time diagnostic test is ensured by the selection of the correct primers and probes, as well as the selection of stringent reaction conditions. Primers and probes sequences were validated with *in silico* analysis. Primers and Probes sequences were designed to specifically identify the gene variants listed in Table B. Primers and Probes sequences were tested for potential homologies with all the gene sequences published by the international gene bank (GenBank®, NIH genetic sequence database) using sequence comparison analysis.
Results: No cross homology was found with other organisms for designed primers and probes

Table B

Gene	Variants
KPC	1 to 17
NDM	1 to 10
VIM	1 to 6, 8 to 38
OXA	48, 162, 163, 181, 204, 232, 244, 245, 247, 370.

2.2 Analytical Specificity

The experimental specificity of the Check-Direct CPE real-time diagnostic test was determined by testing the cross-reactivity with samples containing non-target organisms. 132 carbapenemase-negative strains were used to test the specificity of the Check-Direct CPE real-time test, see bacterial strains listed in Table C.
Results: All isolates tested negative with the Check-Direct CPE assay and the internal control was detected in all samples. The Check-Direct CPE test showed 100% specificity based on the reference strains tested.

Table C

Organisms	#	Organisms	#
Citrobacter freundii	5	*Enterococcus faecalis*	2
Campylobacter jejuni	2	*Klebsiella oxytoca*	1
Enterobacter aerogenes	1	*Klebsiella pneumoniae*	16
Enterococcus casseliflavus	1	*Pseudomonas aeruginosa*	2
Enterobacter cloacae	42	*Staphylococcus aureus*	2
Escherichia coli	51	*Salmonella thypimurium*	1
Pseudomonas mirabilis	3	*Stenotrophomonas maltophilia*	2
Serratia marcescens	1		

3. Analytical Reactivity

To evaluate the analytical reactivity, a retrospective study was performed with 93 bacterial strains of 26 different gram-negative species (Table D). The 93 bacterial strains tested with Check-Direct CPE were previously identified carbapenemase-positive with the micro-array diagnostics test Check-MDR CT103 (Check-Points Heatlth).
Results: All 93 bacterial strains were typed correctly for the targeted carbapenemase genes with the Check-Direct CPE test.

Table D

#	Check-MDR CT103	Check-Direct CPE
19	*KPC*	*KPC*
16	*NDM*	*NDM/VIM*
1	*NDM+OXA-48*	*NDM/VIM+OXA-48*
33	*VIM*	*NDM/VIM*
1	*VIM+OXA-48*	*NDM/VIM+OXA-48*
23	*OXA-48*	*OXA-48*